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## III. Observations on Pelvetia

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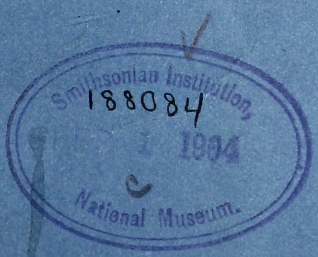


# III. OBSERVATIONS ON PELVETIA.

F. L. Holtz.

*Pelvetia fastigiata* (J. Ag.) DeToni, is a marine alga found distributed along the western coast of the United States and British Columbia. It grows in beds, attached to the rocks, between high and mid tide, and is, therefore, daily exposed to the air for several hours (*Pl. VIII.*). The material studied for this paper was collected by Miss Josephine E. Tilden on Vancouver Island, in June, August, and December, 1901, and was preserved in formalin.

There was originally some doubt in the minds of systematists whether this plant was a *Pelvetia*. It has been called *Fucus fastigiatum* (J. Agardh, Symb., I., 3) and *Fucodium fastigiatum* (J. Agardh, Sp., I., 203). The difficulty of placing it arose from the uncertainty as to the number of eggs it forms in the oögone, and this point was left undecided by DeToni. Dr. W. A. Setchell seems to have been the first to demonstrate the true generic position.*

*External appearance.—Pelvetia* is one of the smaller wracks. It is 10–20 cm. in height, and springs from a disc-shaped holdfast with dichotomous branches repeated till it presents a fascicled appearance. In well-developed plants the stipe branches immediately above the holdfast, and the branches subdivide again but a short distance farther on, so that at first sight there seems to be several fronds arising from the same holdfast. The regular dichotomy near the base may be further confused by adventitious shoots springing from near the base of the main stipe. In the material at hand but one main stipe was observed arising from a holdfast. The front may undergo dichotomy a dozen times before the terminal laminæ are reached. The internodes are longer toward the top. The coördinate branches keep about equal growth, though a few may remain smaller and hence appear like lateral branches (*Pl. VII.*).

*Setchell, W. A. Phyc. Bor. Am., No. 176.

The holdfast is a disc about 1 cm. across, and may be somewhat lobed at its margin, due to protruding masses of cells that are somewhat rhizoid in function, tending to clasp the irregularities of the substratum. The under surface appears slightly rough and pitted.

The mature stipe is elliptical in cross-section, but not winged. It is thicker and rounder at the holdfast, but flattens out into a ribbon-like shape farther up, and widens and thickens slightly toward the top. The average width at the bottom is 3 mm., and at the top 4 mm. It is about 1 mm. thick at the base and 2 to 3 mm. at the top. It is tough and coriaceous below, soft and fleshy above.

The laminal portion is usually two-lobed, and is differentiated externally from the stipe by a rather abrupt thickening and by the fact that it is generally dotted over with the elevated ostioles of the conceptacles, giving it a warty appearance. The lamina is also more translucent than the stipe. The lamina is wedge-shaped; the lobes into which it is divided in its upper half are tapering with rounded points. The laminæ have the softest tissue in the plant. There are usually laminæ in all stages of development, on a main branch, from cylindrical stipe-like laminæ to old, flat, warty, fruiting ones.

The color of the plant is nearly uniformly light brown, the older parts being a little darker, especially the lower stipe. The surface, except on the fruiting lobes, is smooth and shining. The plant is very elastic.

Adventitious shoots may arise on any part of the surface of the plant. They occur chiefly where old wounds have healed over. For instance, where a branch has been torn of, or a lamina cut off, or where incisions have occurred, here may be found proliferations arising as small outgrowths. Sometimes only one may occur, again a dense cluster. Some of these develop into large shoots.

The conceptacles may easily be seen by looking through the translucent lamina toward a strong light. They are thickly scattered over the lamina and its lobes. There is a rude arrangement of the conceptacles in rows running approximately perpendicularly across the axes of the lobes. There are 150 to 200 conceptacles on a lamina. They are developed in the younger tissue at the ends of the lamina lobes. Hence the more mature conceptacles are found some distance from the tip

of the lobe. Occasionally conceptacles are scattered over the stipe. These are generally less mature than those on the lamina above. They may be formed here adventitiously after those of the lamina, or else they may have been formed before or at the same time as those on the lamina and were then arrested in their growth.

The conceptacles cause a small papilla in the surface above them. This can be easily seen with the naked eye, as can also the ostioles themselves, which appear as little pits in the tops of the papillæ. A well-developed plant may have half a dozen main branches and fifty to sixty laminæ.

When placed in fresh water the mucilage of the interior of the plant absorbing the water, causes the laminæ to burst. The distending pith pushes its way out and the cortex curls back, showing a state of tension between interior and exterior. As a result the cortex pulls off from the pith. The conceptacles then appear plainly as little spherical masses projecting from the inner side of the cortex. This intimate union of the conceptacle with the cortex might be taken as evidence of the cortical origin of the conceptacles, which is the case, as will be shown. In *Plate VII* conceptacles are visible on the inside of the cortex in the bursted laminæ.

*Minute anatomy, tissues in general.* — *Pelvetia* shows considerable differentiation of tissues, though not so much as many other algæ, not even so much as some of the other Fucaceæ. *Fucus* shows greater differentiation in having a midrib and air vesicles in addition to the structures possessed by *Pelvetia fastigiata*.

There are three principal tissues in the body of the plant. The epidermis, cortex and pith comprise the main bulk of the body. In the holdfast, however, no real pith cells are found.

*Epidermis.*—The epidermal tissue of *Pelvetia fastigiata* consists of a layer of prismatic cells elongated radially to about twice their shorter diameters, which are about equal. The epidermis is best developed in the stipe and lamina. Seen here in surface view the cells present a roughly quadrangular or polygonal outline. The epidermis is shown in longitudinal and cross-sections in *Figs. 1–3, Pl. IX.* The inner end of the epidermal cells and their radial walls are thin, while their free surface walls are convex outward. The surface of the epidermis is covered with a cuticle, thick and striated. This

cuticle is a common sheath to the whole plant. It is depressed into the crevices between the cells and is therefore wavy in section. It peels off in places. It shows a different, generally weaker, staining reaction from the regular cell wall. The wall underneath the cuticle is thin. The cuticularized epidermis probably is useful in preventing evaporation when the plant is exposed between tides. The hygroscopic nature of the mucilage within no doubt plays a very important part in this respect.

The epidermal cells are densely gorged with chromatophores. These are yellow, highly refracting grains of oval shape. As the function of the epidermis is assimilative as well as protective, the question arises, may not the convex outer walls of the cells aid in condensing the light that is necessary for assimilation (Kerner)?

The epidermal cells have the power of dividing radially and periclinally, cutting off basal cells that are added to the cortex and cause growth in thickness. The division in planes transverse to the axis of the plant provides for the elongation of the plant. This cambium-like nature of the epidermal cells is also seen in the origination of a meristematic layer where a conceptacle is to be produced (*Pl. XI., Fig. 17*), and again the growing point is an epidermal cell (*Pl. X.*).

*Cortex.*—(*Pl. IX., Figs. 1–3, 5.*) Below the epidermis are six or seven rows of cells of varying size and nature, differing more or less from the epidermal and pith cells, and agreeing in a general way in not being greatly elongated and in having a large number of chromatophores. This tissue is the cortex. (The epidermis is by some writers included under this name.) The cells of the cortex are arranged with considerable regularity in vertical, radial and concentric rows. The regularity of shape and size tends to diminish towards the pith.

The row of cells immediately beneath the epidermis is composed of the basal cutoffs from the epidermal cells. They are cuboidal cells of a diameter equal to the width of the epidermal cells. They, like the outer cells, are gorged with color bodies. The second and third concentric rows of the cortex are, in cross section, of equal diameter, but a little larger than the row above. In longitudinal section it is seen that these cells are generally respectively two and four times as long as the basal cells of the epidermis. Some of the cells also show this larger size in cross-section. These cells seem still to have the power

of growth; and to some extent they divide both radially and vertically, but not tangentially. These two rows of cells also have rather thin walls, although there is some thickening at the angles. They are also densely packed with chromatophores.

Below the above-mentioned cells are three or four (five) concentric rows of cells that pass over into the pith on the inner side. Their walls are considerably thickened with a gelatinous substance, which, however, is firmer and denser than that of the pith. These walls stain deeply. The cells of these rows contain color grains but in more loosely disposed masses. The protoplasmic sac is more easily seen around these masses of chromatophores than in the outer cells. These cells have nearly the same radial diameter as the cells in the second and third rows, but are generally twice as wide tangentially and twice as long vertically as those of the third row. There is more or less variation in the number of rows of each of these different sizes of cells. These elements may be diagrammatized as in *Pl. IX.*, *Fig. 5*.

The original walls between these cells thicken as the pith is approached. The cells lose their rectangular shape more and more towards the pith till at last it is sometimes difficult to distinguish them from the more cylindrical pith cells. The cells remain in communication through pits, the cells anastomosing frequently. The longer cells form transverse septa, which are often oblique to the lateral walls. These septa are never thickened, but remain very thin and, to all appearance, allow protoplasmic communication (*Pl. IX.*, *Fig. 12*).

*Pith.* — (*Pl. IX.*, *Figs. 1–5.*) The pith of the stipe and lamina is distinguished by the fact that the cells are separated widely by intercellular jelly, which in the lamina is from two to three times as thick as the diameter of the cells imbedded in it, less thick in the stipe (1–2). The pith is also marked off by the jelly not staining as deeply as the intercellular matrix in the cortex. With some stains, fuchsine for example, the stain may be almost completely removed by washing, leaving the inner wall of the pith cells colored. Pith cells are slightly compressed corresponding with the flattening of the stipe or lamina (*Pl. IX.*, *Fig. 1*). They are nearly as wide as the average width of the cortex cells, and are about as long or a trifle longer than these. They are crossed by delicate septa (*Pl. IX.*, *Figs. 2*, *4*, *13*). Pith cells are joined into vertical rows or filaments

which wind about and intertwine somewhat with each other. These filaments anastomose frequently and are often dichotomously divided (*Pl. IX.*, *Figs. 3*, *4*, *13*). The cells of the pith in the central part of the stipe or blade are of nearly uniform diameter throughout their length and are regular in shape, except at anastomosing plexi and near the cortex where they are subject to distortion in shape and to displacement.

The pith cells contain a few chromatophores collected into a little pellet near the middle of the cell. The protoplasmic contents show up well and numerous refracting grains of reserve material are seen.

The gelatinous intercellular matrix swells up greatly when the plant is placed in fresh water. This causes the lamina to burst open, beginning at the more tender tip of the lobes in the young laminæ. The stipe having a firmer cortex and also proportionately less intercellular gelatine, does not burst, though it swells some.

*Anastomosis and Pits.* — Anastomosis is seen best in the pith cells (*Pl. IX.*, *Figs. 2–4*, *12–14*). Sometimes two filaments will simply be bent toward each other, touching with their convexities (*Fig. 2*). At this point there is no intercellular jelly separating the cells. A thin communicating plate is between the cells in contact, and the protoplasm in these cells sends out branches that meet at the plate. At other times the anastomosing cells send out lateral protuberances, which passing through the jelly, meet and form a pit at the point of contact (*Figs. 4*, *13*, *14*). Probably these protuberances were not formed before the pit was formed, but are the result of the growth and modification of shape of the cells which took place after the pit already was made. Judging from the conditions in the cortical layers, these pits are simply the original fission walls left unthickened at these spots (*Fig. 12*), while at other points the contiguous cells were forced apart by the development of the gelatinous middle lamella between the two cells, however, leaving the cells in contact at the pits. The pits at lateral anastomosing points are smaller than at the ends of the vertical cells. The pits do not stain as deeply as the rest of the cell wall, but this might be due to their greater thinness. They are sharply marked off in the walls of the pith and the inner cortex cells. They are round or oval plates which in optical section are of uniform thickness, not lenticular. They show less definition of

shape as the outer cells of the cortex are approached. Here they appear to be simply the original dividing wall. They can, however, be located by the protoplasm apparently running right through the wall at these places. This apparent communication of the protoplasm of adjoining cells was observed as far out as the second layer of cells below the epidermis. Farther out this could not be seen on account of the chromatophores. But probably even the epidermal cells communicate with each other. The concentrated sulphuric acid test showed that the plates were dissolved as well as the rest of the wall. No positive proof was found that the pits were perforated, no threads of protoplasm having been observed, as would indeed be difficult with the extreme thinness of the plates. But the symmetrical arrangement or attachment of the protoplasm on both sides of the pits leads one to suspect very strongly that there is communication. By plasmolysis the protoplasm draws away from the cell wall at all other points than the pits (*Fig. 13*). It remains attached here and extends in ropes through the cells and seemingly through the pits. The protoplasm often branches to lateral pits (*Fig. 14*). When the pith cells are swollen in fresh water the protoplasm is frequently torn off from one end of the cell, away from a pit, owing apparently to the elongation of the lateral wall as well as the gelatinous matrix. In such cases the pit curves in toward the loosened protoplasm (*Figs. 12, 14*).

Iodine is the most satisfactory stain to use in studying pits. The protoplasm is stained and its attachments may be studied. Pits and anastomosis may be nicely studied by removing some of the protruding pith from a lamina that has burst in fresh water. By flattening the gelatinous mass under the cover glass the pith cells and their pits show up well, even unstained, though better if differentiated with stains for walls and for protoplasm.

*Anatomy of Holdfast, Stipe and Lamina.* — The above matter on the tissues in the body of *P. fastigiata* needs some modification and addition when the holdfast, stipe and lamina are considered separately.

*Holdfast.* — In a vertical section through a holdfast it is seen to be composed of approximately regular, ascending rows of cells; those near the central part more vertical; those near the border of the holdfast curving out as they go down. There is a marked difference between the cells in the middle and those

in the peripheral part. The former are irregularly quadrangular in outline in both vertical and cross-section. Their diameters in cross-section are equal (*Fig. 6*). They are more regularly disposed in rows than the cells in the marginal parts. The cells of the holdfast have walls of good thickness, composed in part of the usual mucilaginous substance.

Taking the central part of the holdfast (*Fig. 6*), the lowest cells are dead and empty and partially disintegrated into mucilage. Clefts arise among the still living cells from this disintegration. Gaps are also caused in this way in the body of the holdfast. Here and there individual cells at the bottom have a disc-like lower surface as if they had a holdfast of their own. The decay of the cells near the central part of the holdfast extends not more than one or two cells deep. The next few rows of cells are slightly flattened parallel with the base of the holdfast. The succeeding rows of cells become gradually elongated in a vertical direction till, at the tenth or eleventh row, a rapid differentiation begins with cells elongated in the vertical direction to two or four times their horizontal diameter. Evidently the stipe begins at this zone.

The peripheral portion (*Fig. 7*), as was stated above, is composed of rows of cells descending obliquely from the axis of the stipe. A vertical section through this part shows that these rows of cells branch dichotomously in the horizontal plane as they go down and outward. The cells decrease in length as one follows the dividing branches, till a zone is reached in what corresponds to the cortex of the stipe. Here the ultimate dichotomous divisions of the main strands form a meristem of small cells. The cells of this meristem run in straight rows perpendicularly to the surface of the holdfast. They are in active division. This meristematic layer enables the holdfast to grow in thickness and also to form the rhizoid-flaps on its edge. It is about eight to ten cells deep. In the specimen examined the basal cells, three to five deep in this part of the holdfast, showed advanced disintegration. The epidermal layer near the lower edge also was in a similar condition. But the cells of this layer are more resisting and persist alive after the two rows beneath are already dead. Probably the mucilage derived from the disintegration of these basal cells is useful in attaching the plant to the substratum.

The cortical part of the holdfast passes without any marked change into that of the stipe. The epidermal cells however are not elongated as much radially as those of the other parts of the surface of the plant. It is covered with a cuticle, thicker than on the stipe or lamina.

Cross-sections of the central part of the holdfast show (*Fig. 8*) that the vertical rows of cells seen on vertical section are not disposed in any regular order. The intercellular substance is not nearly as abundant as in the stipe. Toward the margin of the holdfast the cells show power of dividing. Here we find, interspersed with cross-sections of the vertical rows, sections through cell rows slanting up toward the axis of the plant. Still nearer the outside we come upon the meristematic zone. Here are principally slanting rows of cells dividing dichotomously in the radial direction. These divisions repeat the dichotomy, running directly to the surface.

All the living cells in the holdfast have chromatophores. The central cells contain but few grains, the cortical are crowded with them.

*Stipe.* — But little need be added here to what has been said under tissues in general. The young stipe has a nearly cylindrical structure, with a slight notch on the end where a growing point is situated. No differentiation is noticeable between stipe and lamina. Older stipes become flattened, partly on account of the flattening of the cells parallel with the longer axis of the cross-section, but more on account of the greater growth toward the thin margins. A cross-section of an older stipe shows two principal planes of fission by the arrangement of the cells in rows parallel with the major axis of the section and the other obliquely across this axis. This is especially noticeable in the pith. The cortical cells show a distinctly concentric arrangement (*Fig. 1*).

The only differentiation seen in cross-section is that the pith and inner cortex cells near the ends of the ellipse are somewhat larger than those of the central part. This differentiation however does not even suggest a midrib. Longitudinal sections of the stipe, cut parallel to the flat surface, show a similar appearance, except that the typical pith cells are reached sooner in passing from the surface along the minor axis. The appearance of the cells in both cross and longitudinal sections has been discussed under tissues in general.

*Lamina.*—The general tissues of the stipe and lamina are so

similar that no change is noticeable in passing from one to the other. In the lamina proper, however, the pith cells branch more and the rows of cells have a more meandering course, and there is more anastomosing. The intercellular jelly is developed here more than elsewhere in the plant. Due to this and some to the branching of the cell rows the blade is much thicker than the stipe.

The cortex and epidermis are similar to those of the stipe. The crowding growth of the conceptacles disarranges the orderliness of the cell arrangement in the cortex and epidermis. Cross and longitudinal sections of the lamina resemble those of the stalk closely, except for the differences just mentioned, and for the conceptacles (*Fig. 3* shows a partial cross-section of a lamina).

*The growing point.*—In the tip of the maturer laminæ no definite growing point can be found. There still is some growth and cell division going on here in the outer cortical cells, and in a mature lamina this is probably the youngest and tenderest portion. It is here that the lamina begins to burst when placed in fresh water. Even at the sinus between the lobes of the lamina no growing point can be found in older laminæ. This is the place where the growing point once was. But the growth seems to have stopped here first and continues for a time longer toward the ends of the lobes.

If a young frond is examined, one in which there is as yet no difference between stem and blade, a slight notch or dimple is visible at the top. This notch deepens in older fronds, and if a section is made through the somewhat flattened stipe, parallel with the flat surface and through the axis of the frond, a large apical cell is seen at the base of the sinus (*Pl. X., Figs. 15, 16*). This apical cell is an epidermal cell. It is in the shape of a truncated pyramid, with the truncated end to the top or pointing outward. The apical cell is two or three times as large as the other epidermal cells, and is otherwise markedly distinguished by great richness and granularity of contents, and by the absence of chromatophores. The adjacent cells share these characteristics to a less degree. They show a diminution in the granularity of the protoplasm, and color grains begin to appear in all but the latest cutoff.

The apical cell, as seen in vertical section, cuts off daughter cells in succession, a lateral, then a basal, and then a lateral

on the other side (see diagram, *Fig. 16*). The daughter cells quickly divide again and again, but more frequently in a lateral direction from the apical cell than downward. The cells in these lateral zones divide more rapidly in planes transverse to the axis of the lamina. In this way the zone of most rapid growth extends out laterally and upward from the apical cell and soon grows up ahead of the growing point. As a result there is the bifurcated lobe.

Differentiation into the long pith cells begins only three cutoffs below the apical cell. In the wings it does not begin so soon. The zone of cells in the wings retains its power of fission longer than the cells below the apical cell.

The cells of the epidermis and the cortical zone attain the characteristics of these tissues but a short distance from the apical cell.

The outer cortical cells throughout the plant are capable of dividing and seem to constitute a kind of cambium around the plant. This meristematic nature of the cortex is most highly developed in the lobes of the young lamina near the growing point. It is also well developed where conceptacles form and in the marginal parts of the holdfast.

The cuticular sheath that covers the whole plant is very thick over the delicate growing point, being about as thick as the length of the epidermal cells beneath it, no doubt serving as a protection.

It is customary to speak of the rows of cells in the plant as hyphæ. But when the origin of these cells is considered, that they are derived directly or indirectly from a single apical cell, the idea of their hyphal character seems a little incongruous.

*On the development of the conceptacle.* — As before noted the conceptacles show an intimate connection with the cortex. Sectional views prove the cortical origin and nature of these structures.

The first indication of the beginning of a conceptacle is seen to be the cutting off of a basal layer of cells from the lower end of a few adjacent epidermal cells (*Pl. V., Fig. 17*). These basal cells in turn divide periclinally and radially to form a little pad of meristematic cells beneath the epidermis, around which the cortical cell-rows are deflected. Directly over this mass of basal cutoffs, usually in the center, one or more epidermal cells begin to show signs of disintegration and collapse.

The walls of these cells stain more deeply than those of normal cells, the nuclei disappear and the chromatophores fuse together into a dark mass. The affected cells collapse gradually, beginning at the outer end. Often a little conical remnant of the shrunken cell may be seen on its basal cell. The walls and contents of the disintegrating cells change into a mucilaginous substance.

Thus far my observations agree with those made by F. O. Bower.[1] Bower states that the epidermal cell collapses, but that the basal cell persists, and that it sinks farther and farther into the cavity of the conceptacle, and that the lateral daughter cells of the central basal cell by their division form the lining wall of the cavity. He seeks to limit the disintegration of the epidermis at first to one cell and to make its basal cell the center of the whole process of the development of the conceptacle.

The serial sections made by me for the investigation of this matter do not show that the disintegration is thus confined to one single epidermal cell. Occasionally several will be equally far advanced in decay. Naturally one or the other of these may decay more quickly than the rest, producing thus a line of weakness and apparently a central axis about which the other decaying cells are grouped.

Again, it was not found that the basal cell or cells of the disintegrating epidermal cells persisted. On the contrary, they and several rows of cells below, perhaps five or six, share in this disintegration. It was frequently possible to make out the remains of the disintegrating cells in the mucilaginous mass to which they changed, and with which the cavity formed by their collapse was filled.

Neither did it appear that the basal cutoffs of the epidermal cells produced lateral daughter cells to line the cavity. It did appear that they divided chiefly periclinally and somewhat radially, forming five or six rows of meristematic cells, the outer rows disintegrating and forming the cavity; the deeper ones persisting and finally forming the inner wall of the conceptacle and giving rise to paraphases and the reproductive organs. Bower shows figures like *19*, *Pl. XI.*, in which the two cells, *b* and *c* on either side of the central basal cell *a*, might suggest that they were the lateral daughter cells of this basal cell. But

[1] Bower. Development of the Conceptacle in Fucaceæ. Qr. Jr. Mic. Sci. 36. 1880.

sections like *Fig. 21* are met with in which it is clearly seen that these lateral basal cells are not the daughter cells of the central basal cell *a*, but that they are the basal cutoffs of the epidermal cells above them. They are, therefore, coördinate with the basal cell *a*. The cells *e* and *f* are later cutoffs which the epidermal cells *g* and *h* succeeded in cutting off before becoming affected by decay. On account of less resistance from the cavity than on other sides these lateral basal cells grow usually in the shape shown in *Fig. 19*.

To summarize my conclusions on this point, the conceptacle originates by a few contiguous epidermal cells cutting off basal cells, *Fig. 17*, which are meristematic, dividing principally periclinally into half a dozen or more tiers of cells. Directly over this meristematic mass of cells, whether by the tension produced by the growth of the cells below, or otherwise, one or several epidermal cells begin to show signs of decomposition. The disintegration proceeds and the cells collapse (*Figs. 19* and *21*), and a cavity is begun. The disintegration spreads to neighboring epidermal cells and to the cells in the meristem below (*Figs. 21*, *23* and *25*). By their decay the cavity is enlarged. The deeper and marginal cells in the meristematic mass do not disintegrate, but in the end make the inner wall of the conceptacle, and give rise to paraphases and reproductive organs (*Figs. 27*, *28*, *29*). The mucilaginous remains of the decayed cells for a time fill the cavity, or protrude from its mouth, or close the mouth as a stopper. The diagrams, *Figs. 18*, *20*, *22*, *24*, *26*, corresponding respectively to *Figs. 17*, *19*, *21*, *23*, *25*, illustrate how it is possible to explain the development of the conceptacle without using Bower's central, persisting, basal cell theory. It is not probable that the development of the conceptacle in *P. fastigiata* is different from that in the closely related plants which he describes. Since this work by Bower is the principal reference we have on the development of the conceptacle in the Fucaceæ, and is generally quoted, it would be profitable for others to repeat these investigations.

Finally the disintegration stops, a healthy surface layer of cells then lines the cavity and the dead and mucilaginous cells are cast off into the cavity. Meanwhile the unaffected epidermal cells continue to divide and form their basal cells which pass into the cortex. This new cortical growth stops abruptly at the conceptacle. In this way the cavity is deepened and a

neck is formed to the cavity, this neck being composed of epidermis-like cells. The original cortical rows are at first slightly deflected around the forming cavity, but later become deeply invaginated and thus aid in the deepening of the conceptacle. The cells of these layers become flattened and lenticular in shape, and are arranged in concentric layers, three to five deep, around the cavity thus forming a basket-like receptacle. The cells on the side toward the cavity are thin-walled and small, the outer cells are larger and have more intercellular jelly (*Pl. XI., Figs. 27–29*).

The cavity of the conceptacle is generally nearly spherical. Occasionally it is oval in shape with the longer axis in various directions. Where several conceptacles occur close together, there may be considerable distortion in their shapes.

The cortex over the conceptacle is slightly elevated by the growth of the conceptacle, but is gently curved again into the ostiole. The angle between the epidermis and the conceptacle is filled in with rather irregularly disposed cortex cells belonging to the deeper strata. The pith is sharply marked off from the flattened cortical cells around the conceptacles.

The mucilaginous remains of the disintegrated cells stay within the cavity for a considerable time, even till the reproductive organs form. Shreds and layers of this mucilage may also be found outside the conceptacle around its mouth. Frequently it closes the neck of the conceptacle like a stopper (*Fig. 27*). It seems to be finally partly absorbed and partly extruded by the paraphyses.

Bower thinks that the protrusion of the conceptacle into the pith is caused by the turgidity of the conceptable when filled and stoppered with the mucilaginous contents, the bulging being rather toward the softer and more yielding pith than toward the more rigid cortex, though even here it is noticeable. This explanation is insufficient, as it hardly seems possible that the conceptacle is closed tightly enough for the purpose, and especially since the greatest swelling of the conceptacle into the pith is in the later stages when the cavity has already begun to discharge or absorb the jelly and is no longer completely filled nor tightly closed. The principle of the arch might help to explain this protrusion of the conceptacle. As the cells in the wall of the conceptacle grow and multiply the arch which they form would create a distinct pressure on the surrounding tissue.

*Paraphyses.* — When the disintegration of the cells to form the conceptacular cavity is about finished, and while masses of mucilage still encumber the cavity, the first appearance of the paraphyses can be observed (*Pl. V., Fig. 27; Pl. XII., Fig. 38*). At this time the conceptacle is lined with one to three layers of thin-walled, ovally flattened cells, which are devoid of chromatophores or have only a few minute ones. The cells near the ostiole have more color bodies. These cells are filled with a granular protoplasm like the apical cell, though not so richly. The walls of these cells do not stain as deeply as the other cortical cells. The granularity referred to is evidently associated with activity in cell division.

Paraphyses arise as protuberances on the inner wall of some of the cells lining the conceptacle cavity. These protuberances may in young conceptacles project halfway across the cavity before they are cut off by a wall from their basal cells. The paraphyses appear at first in the lower half of the conceptacle. They very soon, even before they are cut off from their basal cell, begin to turn toward the ostiole.

As stated, the paraphyses in the main portion of the conceptacle arise as lateral buds from the cells in the wall of the conceptacle. The paraphyses at the upper end around the ostiole appear to form somewhat differently. They look as if they consisted of the unravelled or loosened cell rows of which the conceptacle wall is composed, and which crop out in the region near the top of the cavity (see *Fig. 29*).

As the paraphyses develop their end cells especially divide, though lower cells may do the same. The protoplasm remains in communication between cells. The protoplasm is slightly granular, nearly devoid of color bodies, except the end cells of the paraphyses about the ostiole. These are well provided with chromatophores, from which it would appear that their function is in part assimilative. Mature paraphyses consist, in the lower part of the conceptacle, of four or five cylindrical cells of almost uniform diameter. The end cell is tapering. The cells are about two or three times as long as wide. The paraphyses in the upper part of the conceptacle are more slender and their cells are shorter and more numerous, eight to ten.

The paraphyses are very numerous in a conceptacle. They are especially numerous and crowded at the top, though they are arranged here in regular, parallel order. In a few cases

paraphyses were observed projecting out of the ostiole, but not very much, only two or three cell lengths.

*Reproductive organs.* — The oögonia and the antheridia appear at about the same time. *Pelvetia fastigiata* has hermaphrodite conceptacles, and it is impossible to say that the oögonia or antheridia have special parts of the conceptacle on which to grow. Both may be found anywhere, except that the antheridia do not seem to develop as close to the ostiole as the oögonia sometimes do. Both organs arise in the same way as paraphyses, as buds from the cells that line the conceptacle.

*Oögonium.* — The oögonium may be recognized from the beginning by the fact that the cell which forms it from the first has darker contents than the rest of the cells in the conceptacle wall (*Pl. XII., Fig. 30*). The young oögonia also are darker. The contents of the oögonial mother cell are composed of a very granular protoplasm. The oögone arises as a swelling along the whole free surface of the mother cell. Paraphyses and antheridial hairs do not occupy so much of the free wall of the mother cell. In other words, they start as mere slender buds. After extending into the conceptacle a distance a little greater than the thickness of the mother cell, a dividing wall is laid down, thus forming the oögone and its basal cell (*Figs. 31* and *32*). This wall is evidently porous, as the protoplasm of both cells seems to communicate through it. The pedicel for some time retains the opacity of its contents but later becomes more like the other cells in the conceptacular wall.

The oögonial cell continues dark, increasing in opacity as it matures. This fact, together with the other fact that the fixing and preservation in formalin is not a good way to prepare these tissues for cytological study, in truth seems to make staining more difficult. For this reason it has been impossible to carry out the study of the development of the oöspheres in a satisfactory manner. It was found, however, that if the sections were bleached from fifteen to twenty minutes in chlorine gas, stained in hæmatoxylin for twenty-four hours, and washed in acid alcohol till the stain of the other tissues was nearly removed, then the nuclei of the oögones could be seen. Methyl violet and acid alcohol also brought out the nuclei. In younger and more transparent oögones the nuclei can be made out without bleaching. In this way the oögone was traced from the uninuclear to the four-nuclear stage (*Figs. 31–36*). Thuret states

that *Pelvetia* oögones divide into eight nuclei and that six of these are afterwards destroyed. Not more than four nuclei could be seen in the material studied.

The ripe oögone contains two eggs. A delicate transverse partition is laid down across the middle of the oögonial contents. Each egg is hemispherical or round-conical in shape. The lower one is often more pointed than the other. Nuclei could not be distinguished with definiteness.

The oögone increases rapidly in size, swelling to an oval or pear-shaped mass which surrounds itself with a thick gelatinous wall (*Figs. 36* and *37*). The oögonial wall is at first not different from that of the basal cell, but it soon thickens and becomes gelatinous so that it swells in water. This thickening continues till in the older oögones the swollen walls present the appearance shown in *Fig. 37*. Stratification is sometimes seen in this wall.

In dehydrating specimens this gelatinous wall splits into two layers, a thin outer layer, and a thicker, firmer, more densely staining one. These layers often remain in contact at different points and generally at the base where both layers are thin (*Fig. 36*). These two layers are the exochite and meso(endo)chite of Farmer and Williams.[1] From their account it would seem that this double-layered condition is the normal. The observations in this case, however, showed that the division of the oögone wall into two layers was unnatural. For nothing like it was observable in sections mounted in water or glycerine. The splitting is probably due to the tensions set up in the dehydration and the thicker mesochite layer is probably formed by the shrinking of the gelatinous middle substance upon the inner layer of the wall, therefore, being denser and appearing more intensely stained. A similar thing is noticeable everywhere in the dehydrated and stained pith.

*Antheridia.* — It is generally possible to find oögonia in any section made through a mature conceptacle. The antheridia are often much scarcer, and search has sometimes to be made through several sections before they are found. There is, however, probably no conceptacle entirely without them. On the other hand, some conceptacles contain a great abundance of antheridia crowded in bunches among the oögones and para-

[1] Contribution to Our Knowledge of the Fucaceæ: Life History and Cytology.

physes. The antheridia are more numerous in the lower half of the conceptacle.

It is usually stated that antheridia in different Fucaceæ develop on branching hairs. This also is the general rule here. It is not necessarily always the case through, for antheridia can frequently be found not on branching hairs, but on simple pedicel cells (*Pl. XII.*, *Fig. 40*). The basal cells sometimes divide and later give rise to branches.

The antheridial hairs arise as papillæ or buds on the walls of the cells that line the conceptacle (*Fig. 39*). These papillæ are soon cut off by transverse walls. The outer cell elongates, divides and the lower of the two cells thus formed sends out a lateral bud near its upper end, which is later cut off by a partition. This process may be repeated several times along the main axis and the branches till a branching growth, not very dissected, is produced (*Figs. 39, 40, 41*).

The protoplasm of contiguous cells is in communication. Few minute chromatophores are found in these branching hairs. The granularity of the protoplasm is not very great as compared with that in the cells which develop the oögones.

Some of the end cells of the branching hairs increase in size, the nucleus divides into two, four, eight, etc., till about sixty-four nuclei are formed, so it is stated by authorities. In the material studied not so many could be counted, or estimated, only about forty. The nuclear division begins early, and different stages are all illustrated in the same section. The nuclei become somewhat smaller by successive division, this being especially noticeable in the earlier stages.

The antheridial cells are at first slender and somewhat pointed, but as the division within continues the cell becomes more and more rounded, usually oval in outline, slightly tapering at the top. The wall of the antheridium is at first thin. It soon thickens and becomes capable of swelling greatly (*Figs. 40* and *41*). The cell contents at first communicate with the basal cells, but later round up and draw away from the dividing wall. The spermatozoids stain quickly. They contain minute chromatophores. From one to six antheridia were observed on a single branching hair.

The plants studied for this paper were collected at the Minnesota Seaside Station by Miss Josephine E. Tilden, of the University of Minnesota. Thanks are due to her for them, and also for helpful suggestions given to the writer.

*Methods: fixing and mounting fluids.* — Material fixed and preserved in formalin was employed. This was washed in 30 per cent. alcohol, as fresh water alone caused injurious swelling of the laminæ. The material was then passed into higher per cents of alcohol to harden and dehydrate. The complete dehydrating seems to cause tensions in the body of the plant resulting in tearing apart of pith cells, the intercellular jelly giving way. But occasional very perfect pith sections may be thus obtained nevertheless, and by comparing with sections cut from 70 per cent. or 80 per cent. alcoholic material and mounted in water or glycerine the nearly natural appearance of these cells can be observed.

Most of the drawings were made from dehydrated material, and must therefore be somewhat unnaturally contracted. Where water or glycerine mounts were made and drawings from them, it is so indicated in the notes explanatory of the plates. The gelatinous walls swell greatly in glycerine (as compared with alcohol) but as the cross-sections of the lamina and stipe, for instance, have practically the same dimensions as the formalin material it may be assumed that the glycerine mounts give a truer picture of the tissues than do the balsam mounts.

*Sectioning.* — Most of the sections, especially the serial sections illustrating conceptacle development and the growing point, were made with a microtome from material imbedded in paraffine. After the work of hardening is once started this method is probably as rapid as any where imbedding is necessary. Some sectioning was done with a hand microtome, the 75 per cent. or 85 per cent. material being held in a pith clamp. Such sections were mounted generally in glycerine. These sections however showed a tendency to curl more than paraffine sections.

*Staining.* — A variety of stains were tried. Many of the ordinary wall stains proved entirely ineffectual. At length the following stains were selected as the best.

Fuchsine and methyl violet is perhaps the most generally useful. This mixture stains quickly and deeply. Washing cautiously in acid alcohol brings out different effects. Only a little washing leaves the gelatinous matrix slightly stained, the inner walls stain deeply, while the cell contents again take a slight coloring. Differentiation is brought out nicely, generally by more washing, in the conceptacular parts. The granular

inner cells of the conceptacle, the paraphyses, oögonia and antheridial hairs stain light red, the deeper cells of the conceptacle purplish, the pith cell walls dark red, while the color is all washed out of the matrix. Differentiation is also produced between different cortical layers, epidermis and cuticle and pith.

Methyl blue alone is a fairly good wall stain, but must be washed with care or it will all wash out. It also stains the chromatophores deeply. In this way the nuclei in the not too opaque oögonia may be located, they shining through as lighter areas in the dark mass of the oögone. Methyl violet is a quick stain. Over-stain and wash out as desired.

Bismarck brown was a very satisfactory stain for mapping out cell structure distinctly. It stains the inner wall of the cells (dark) brown, and the pith yellow. It is also useful in studying the structure of the conceptacle, the gelatinous sheath around the oögone being nicely brought out. It is a quick stain.

It may either be used dilute and allowed to stain longer, or more concentrated and then washed out till desired effect is obtained. Either way is good.

Hæmatoxylin dyes are better than carmine for nuclei. The most satisfactory results were obtained by bleaching the sections in chlorine gas for ten to fifteen minutes, then staining from one to two days in hæmatoxylin, then washing out till the walls and matrix were nearly clear, while the nuclei retained the stain longer. The chromatophores stain in hæmatoxylin and thorough washing is necessary to make the nuclei appear. Delafield's hæmatoxylin was found a good kind. The best effect was obtained by using a hæmatoxylin (brand unknown) that had been kept in solution at least ten years.

Iodine is very satisfactory for staining the protoplasm, and is very helpful in studying the pits between the cells, and in studying the contents of the conceptacular organs.

The staining was done on the slide. Rather concentrated dyes were employed. For alcoholic solutions of dyes 70 per cent. alcohol was used.

## Description of Plates.

### Plate VII.

Photograph of single plant of *Pelvetia fastigiata*. Shows holdfast, bifurcation of stipe and of laminæ. The warty appearance of some older laminæ is due to papillæ caused by conceptacles. Several laminæ have burst open through absorption of water, and show the conceptacles on the inner side of the cortex. About one half natural size.

### Plate VIII.

Photograph showing bed of *Pelvetia fastigiata* on the rocks at Port Renfrew, exposed at ebb tide. This photograph was taken by C. J. Hibbard for the Botanical Department of the University of Minnesota.

### Plate IX.

Anatomical detail: All drawings were made with the aid of camera lucida, diagrams excepted. All on this plate about × 250.

1. Shows part of the cross-section of stipe from epidermis to center of stipe.

2. Longitudinal section of stipe, showing cells with protoplasm, nuclei and chromatophores. The protoplasmic connection between ells is indicated. The cuticle covers the epidermis.

3. Cross-section of lamina. Shows the large amount of intercellular jelly in the pith region.

4. Longitudinal section of lamina, showing anastomosing pith cells. The space between them is filled with intercellular jelly.

5. Diagram of longitudinal and cross-section of stipe or lamina. Shows radially elongated epidermal cell. Beneath this are six or more rows of cortex cells and beyond these the pith. The cortex is shown arising as a basal cut-off of the epidermis. Previous cut-offs are shown in different stages of growth and division. Growth is principally in periclinal and longitudinal directions. The outer cells are more regularly rectangular, but become more rounded on the edges and corners toward the pith. The inner cortex rows finally become modified into long, cylindrical pith cells. The cells are separated farther by intercellular jelly as the pith is approached.

6. Vertical section through central part of holdfast. The lower part is shaded to show that the cells are dead, elsewhere also where such cells occur. The basal cells are flattened. The walls of the dead cells are gelatinous. The cells are shown with chromatophore masses to help indicate division. In the upper part the cells are elongated. Here the stipe begins.

7. Vertical section through periphery of holdfast, showing direction of cell rows. Also shows meristematic character of cells near edge. Disintegrating cells are shaded.

8. Cross-section through central part of holdfast.

9. Cross-section through holdfast nearer to the edge of disc.

10. Edge of holdfast, in cross-section, showing division of cells in this region.

11. Diagram to illustrate transverse and vertical dichotomy of the cells in the peripheral part of holdfast.

12. Diagrammatic view of cross-section of stipe or blade to illustrate protoplasmic communication in epidermal and cortical region. The chromatophores have been left out. Shows increase of intercellular jelly toward pith, crowding cells apart, except at the pits, showing formation of protuberances from adjoining cells.

13. Pith cells showing protoplasmic contents, nuclei and chromatophores. Shows end plates apparently permitting protoplasmic communication.

14. Shows lateral pits at anastomosing point of two pith cell rows.

## Plate X.

Anatomical detail.

15. Growing point with apical cell dividing in two lateral and a basal plane, rapid multiplication of cells in the lateral regions; less rapid in the axial region. Rapid differentiation into pith in axial region; less rapid in the wings. Illustrates mode of bifurcation, × 375.

16. Same in diagram.

## Plate XI.

Anatomical detail. Development of conceptacle, × 300.

17. Beginning of conceptacle. Shows basal cutoffs and disintegrating epidermal cells above them.

18. Same in diagram.

19. Later stage. Shows collapse of a central epidermal cell, remains of same appear as small cone on basal cutoff. Other epidermal cells are disintegrating, having however first succeeded in again cutting of a basal cell each, which have grown obliquely into the cavity formed by collapsed cell.

20. Diagram of same.

21. Later stage, more epidermal cells affected. Cavity filled with mucilage.

22. Diagram of same.

23. Later stage, basal cells disintegrating. Shows division going on in meristematic layer beneath.

24. Diagram of same.

25. Later stage. Shows cavity deepened, filled with mucilaginous remains of cells.

26. Diagram of the same.

27. Young conceptacle. Disintegration of cells has ceased. Their mucilaginous remains act as a stopper to the conceptacle. The neck is being formed by new layers of subepidermal cells. Paraphyses are beginning on the wall.

28. Later stage. Paraphyses and young oögonia have sprung from the cells lining the cavity. Chromatophores abundant near mouth, very few or small in lining cells. Cells in lining of cavity rich in protoplasm.

29. Nearly mature conceptacle with paraphyses, oögonia and antheridial branching hairs. Cells in wall of conceptacle have become flattened.

## PLATE XII.

Anatomical detail. Reproductive organs and paraphyses, × 250.

30. Beginning of oögone and paraphysis. Oögone cell densely granular. Paraphysis begins as a more slender protuberance of cell in conceptacular wall.

31. Later stage. Basal cells have been cut off by oögone and paraphysis.

32. Similar. An oögone anlage preparing to form pedicel cell. Paraphyses elongating and dividing.

33. Nucleus of young oögone dividing in two. Protoplasm still in communication with that of basal cell.

34. Later stage, another division of nuclei. The protoplasm of the oögone has been forcibly torn away from that of basal cell, probably in dehydrating process.

35. Oögone in four-nucleated stage.

36. Four-nucleated oögone showing thickening of wall and separation of wall into thin exochite and thick gelatinous mesoendochite. The two layers still adhere in places. Wall remains thin at basal pit.

37. Mature oögone with two eggs. Was mounted in glycerine which caused swelling of walls.

38. Young conceptacle showing origin of paraphases as protuberances from cells lining cavity of conceptacle.

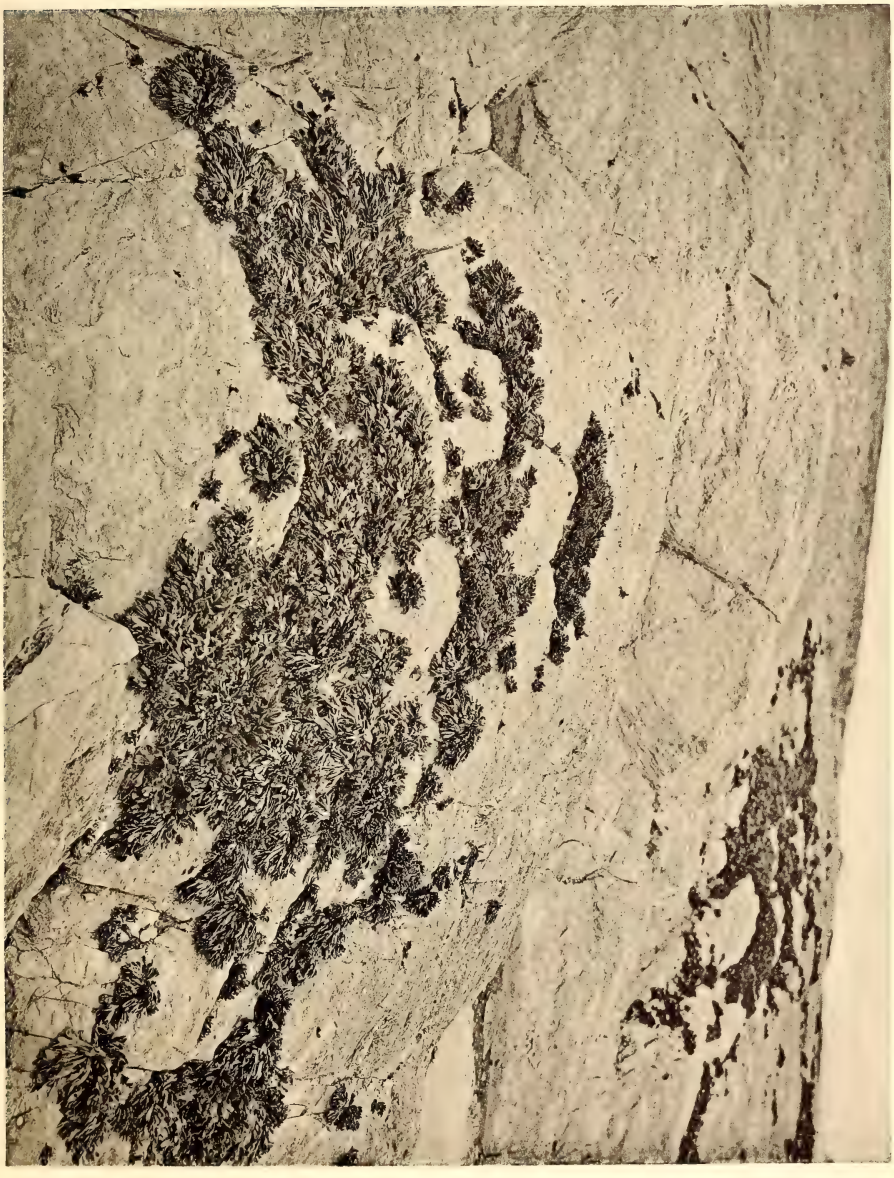
39. Young branching hairs, showing mode of branching.

40. Bottom of nearly mature conceptacle, showing oögone, paraphyses and branching hairs with antheridia. Antheridial protoplasm is shown in different stages of division.

41. Single branching hair in glycerine. Antheridia with sperms and swollen walls.

PLATE VII.

HELIOTYPE CO., BOSTON.



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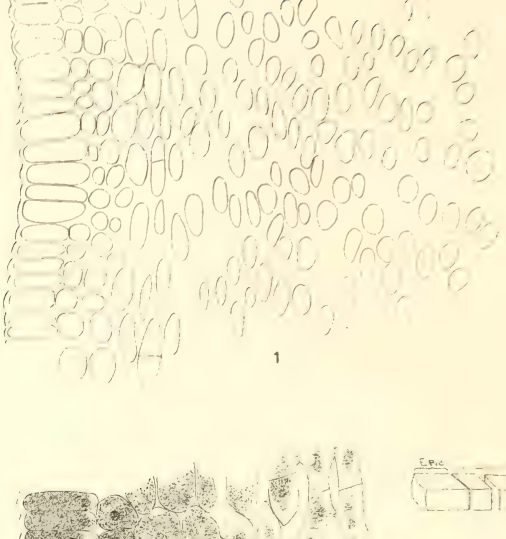

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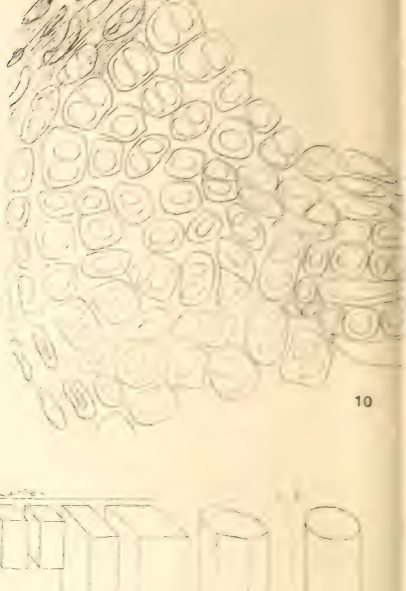

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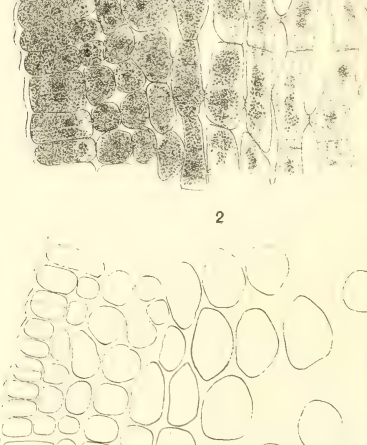

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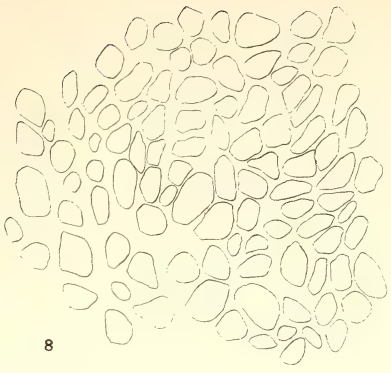

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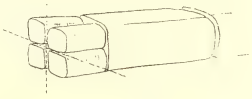

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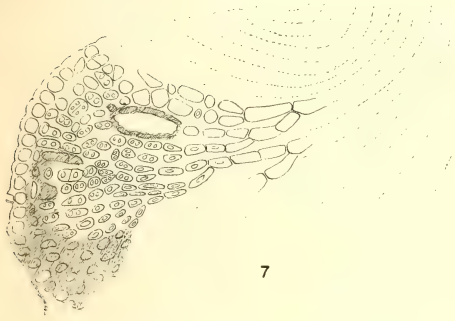

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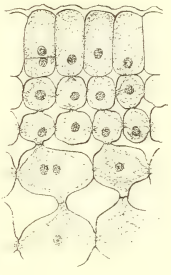

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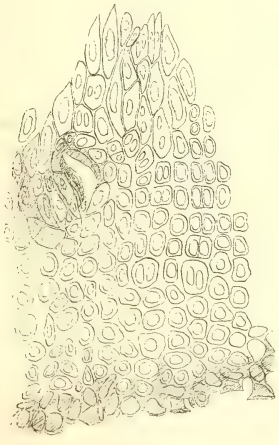

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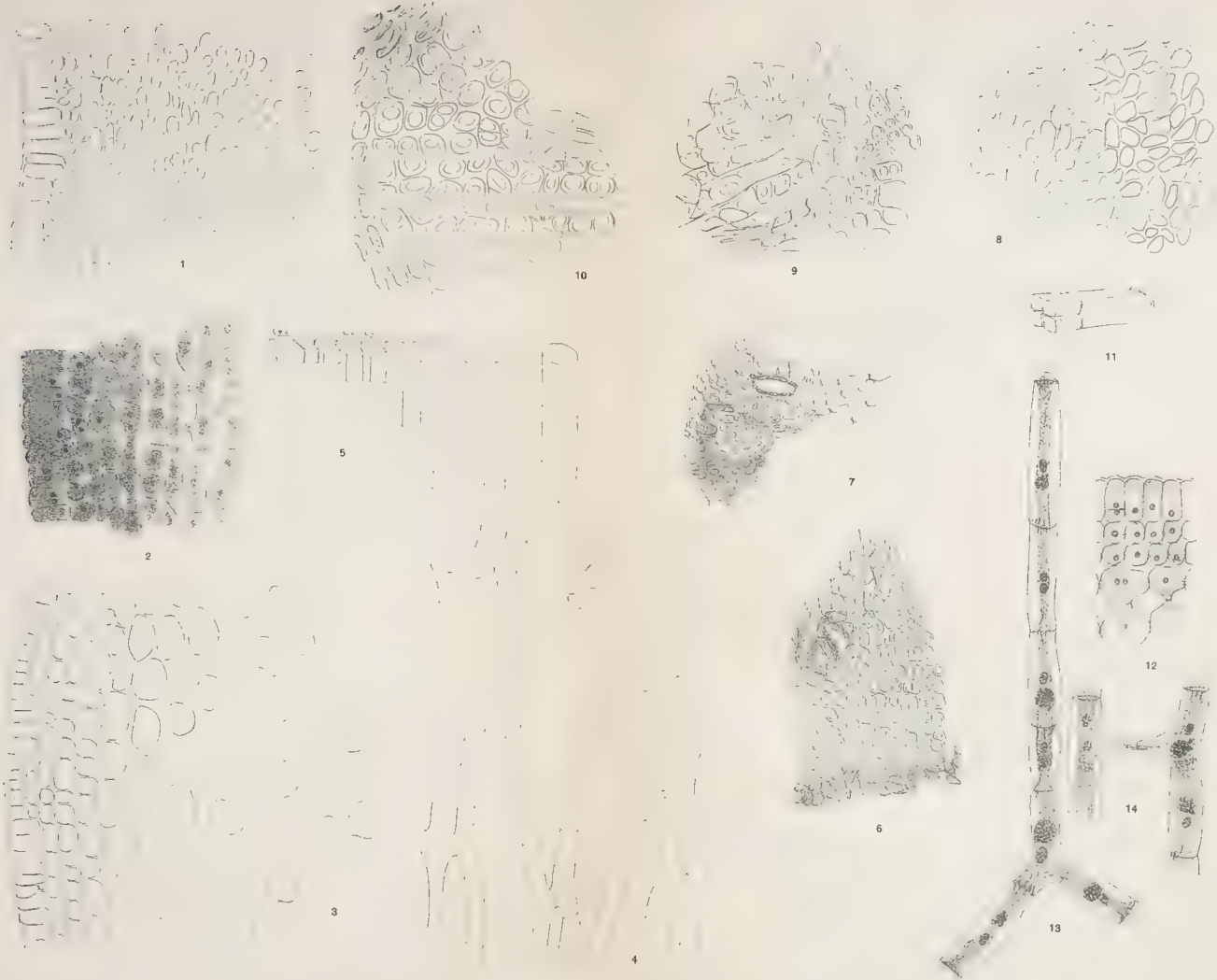


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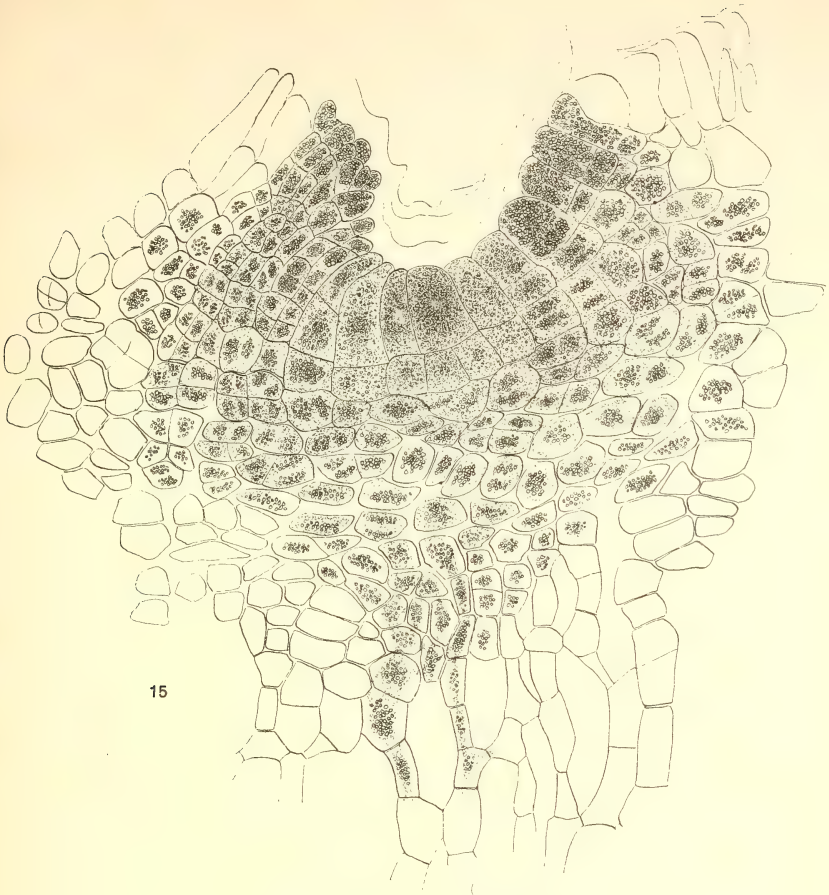

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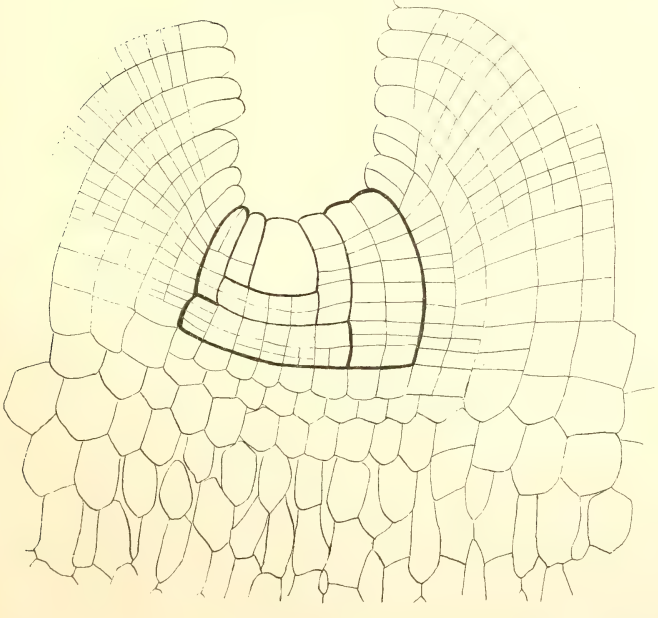

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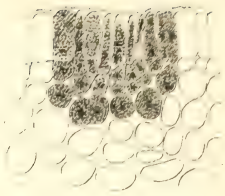

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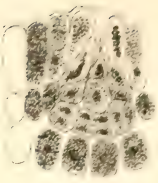

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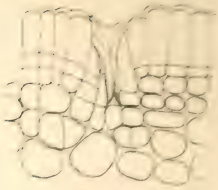

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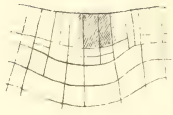

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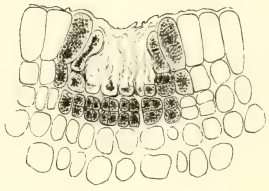

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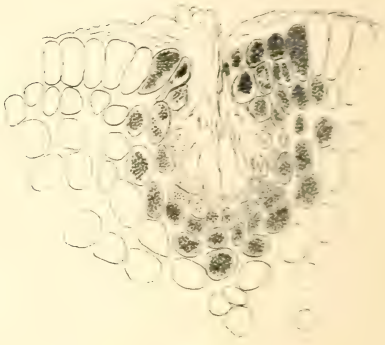

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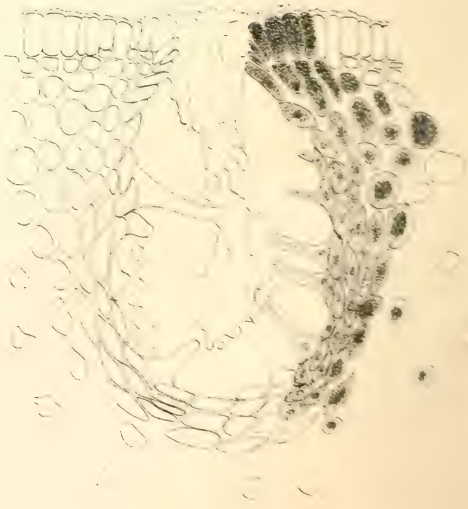

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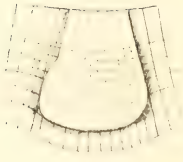

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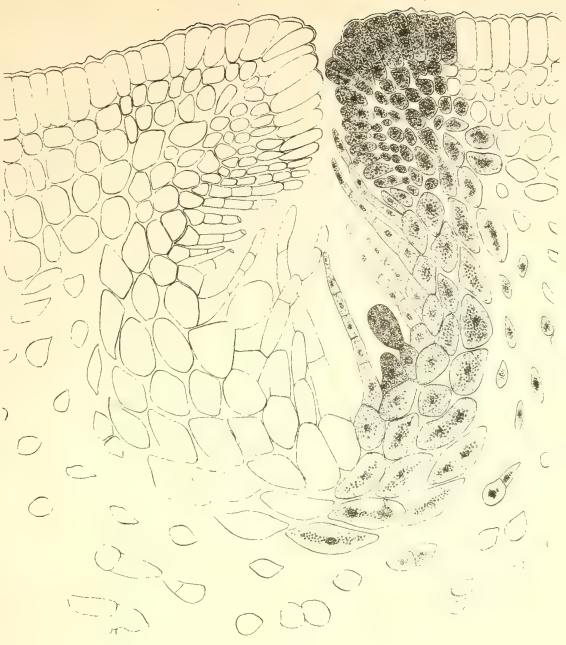

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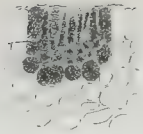
OSTON.



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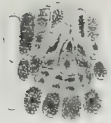

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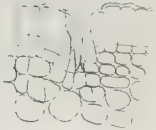

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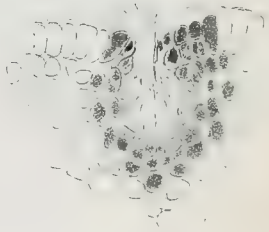

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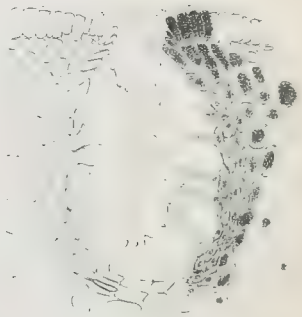

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PLATE XI.

HELIOTYPE CO., BOSTON.

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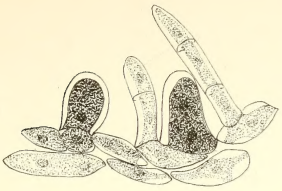

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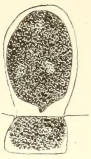

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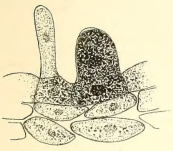

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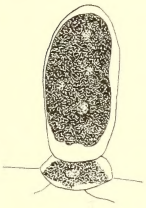

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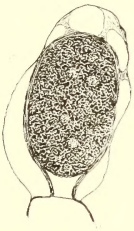

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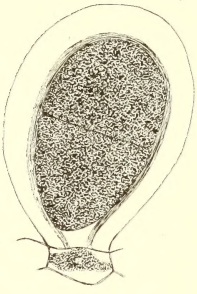

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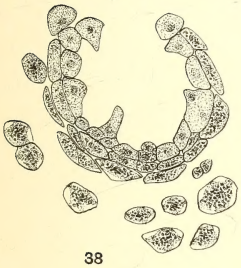

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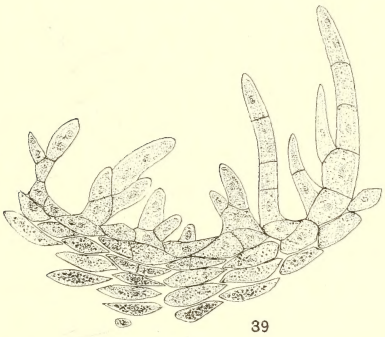

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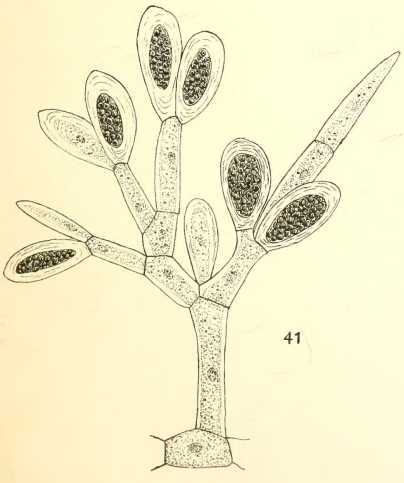

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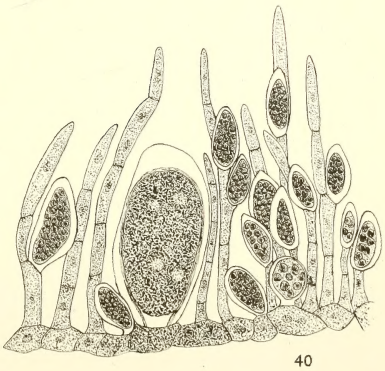

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PLATE XII.

HELIOTYPE CO., BOSTON.